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(71) Applicant *(for all designated States except US)*: **APPLIED MOLECULAR EVOLUTION, INC** [US/US]; 3520 Dunhill Street, San Diego, California 92121 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ALLAN, Barrett, W.** [US/US]; 542 San Dieguito Drive, Encinitas, California 92024 (US). **MARQUIS, David, Matthew** [US/US]; 2106 Meadowgreen Court, Encinitas, California 92024 (US). **TANG, Ying** [CN/US]; 7179 Park Village Road, San Diego, California 92129 (US). **WATKINS, Jeffry, Dean** [US/US]; 3442 Fortuna Ranch Road, Encinitas, California 92024 (US).

(74) Agents: **WISKERCHEN, MaryAnn** et al.; ELI LILLY AND COMPANY, P. O. Box 6288, Indianapolis, Indiana 46206-6288 (US).

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(54) Title: FC REGION VARIANTS

(57) Abstract: The present invention provides polypeptide Fc region variants and oligonucleotides encoding Fc region variants. Specifically, the present invention provides compositions comprising novel Fc region variants, methods for identifying useful Fc region variants, and methods for employing Fc region variants for treating disease.

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Fc REGION VARIANTS

FIELD OF THE INVENTION

The present invention relates to polypeptide Fc region variants and oligonucleotides encoding Fc region variants. Specifically, the present invention provides compositions comprising novel Fc region variants, methods for identifying useful Fc region variants, and methods for employing Fc region variants (e.g. for treating disease).

BACKGROUND OF THE INVENTION

There are five types of immunoglobulins in humans. These groups are known as IgG, IgM, IgD, IgA, and IgE, and are distinguished based on the isotypes of the heavy chain gene (gamma, mu, delta, alpha, and epsilon respectively). The most common isotype is IgG, and is composed of two identical heavy chain polypeptides and two identical light chain polypeptides (See, Figure 1). The two heavy chains are covalently linked to each other by disulfide bonds and each light chain is linked to a heavy chain by a disulfide bond (See, Figure 1). Each heavy chain contains approximately 445 amino acid residues, and each light chain contains approximately 215 amino acid residues.

Each heavy chain contains four distinct domains that are generally referred to as variable domain (VH), constant heavy domain 1 (CH1), constant heavy domain 2 (CH2), and constant heavy domain 3 (CH3) (See, Figure 1). The CH1 and CH2 domains are joined by a hinge region (inter-domain sections) that provides the Ig with flexibility. Each light chain contains two distinct domains that are generally referred to as the variable light (VL) and the constant light (CL).

The variable regions of the heavy and light chains directly bind antigen and are responsible for the diversity and specificity of Igs. Each VL and VH has three complementarity-determining regions (CDRs, also known as hyper variable regions). When the VL and VH come together through interactions of the heavy and light chain, the CDRs form a binding surface that contacts the antigen.

While the variable regions are involved in antigen binding, the heavy chain constant domains, primarily CH2 and CH3, are involved in non-antigen binding functions. This region, generally known as the Fc region, has many important functions.

For example, the Fc region binds complement, which may trigger phagocytosis or complement dependent cytotoxicity (CDC). The Fc region also binds Fc receptors, which may trigger phagocytosis or antibody dependent cellular cytotoxicity (ADCC). The Fc region also plays a role in helping to maintain the immunoglobulin in circulation and interacts with protein A, which is commonly used to purify immunoglobulin.

There has recently been an effort to improve the immunogenic qualities and antigen binding characteristics of antibodies. For example, monoclonal, chimeric and humanized antibodies have been developed for immunotherapy. Examples of antibodies that have been approved for human immunotherapy, with the corresponding disease, include: RITUXAN (lymphoma), SYNAGIS (infectious disease), ZENEPAX (kidney transplant), REMICADE (Crohn's disease and rheumatoid arthritis), HERCEPTIN (breast carcinoma), and EDRECOLOMAB (colon cancer). However, there are many antibodies that have entered clinical trials that have failed to receive approval due to lack of efficacy or other associated problems.

What is needed, in order to improve the efficacy and speed up approval of additional therapeutic antibodies, are compositions and methods for altering Fc regions to generate variant polypeptides with improved properties.

SUMMARY OF THE INVENTION

The present invention provides polypeptide Fc region variants and oligonucleotides encoding Fc region variants, and portions thereof. Specifically, the present invention provides compositions comprising novel Fc region variants, methods for identifying useful Fc region variants, and methods for employing Fc region variants.

In some embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 247 in the Fc region. In particular embodiments, the variant comprises an antibody (e.g. an anti-CD20 antibody). In preferred embodiments, the amino acid modification is P247L.

In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 251 in the Fc region. In particular embodiments, the variant comprises an antibody (e.g. an anti-CD20 antibody). In preferred embodiments, the amino acid modification is L251F.

In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 256 in the Fc region. In particular embodiments, the variant comprises an antibody (e.g. an anti-CD20 antibody). In preferred embodiments, the amino acid modification is T256M.

In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 268 in the Fc region. In particular embodiments, the variant comprises an antibody (e.g. an anti-CD20 antibody). In preferred embodiments, the amino acid modification is H268E.

In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 280 in the Fc region. In particular embodiments, the variant comprises an antibody (e.g. an anti-CD20 antibody). In preferred embodiments, the amino acid modification is D280A.

In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-

mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 330 in the Fc region. In particular embodiments, the variant comprises an antibody (e.g. a anti-CD20 antibody). In preferred embodiments, the amino acid modification is A330K.

In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 332 in the Fc region. In particular embodiments, the variant comprises an antibody (e.g. an anti-CD20 antibody). In preferred embodiments, the amino acid modification is I332E.

In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 339 in the Fc region. In particular embodiments, the variant comprises an antibody (e.g. an anti-CD20 antibody). In preferred embodiments, the amino acid modification is A339T.

In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates depletion of target cells (e.g. B cells) in a whole blood assay more effectively than the parent polypeptide and comprises at least one amino acid modification at position 378 in the Fc region. In particular embodiments, the variant comprises an antibody (e.g. an anti-CD20 antibody). In preferred embodiments, the amino acid modification is A378D.

In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 440 in

the Fc region. In particular embodiments, the variant comprises an antibody (e.g. an anti-CD20 antibody). In preferred embodiments, the amino acid modification is S440Y.

In some embodiments, the present invention provides a peptide (containing the P247L amino acid modification) comprising the sequence shown in SEQ ID NO:15. In other embodiments, the present invention provides a nucleic acid sequence encoding a CH2 region with the P247L modification (e.g. SEQ ID NO:40). In some embodiments, the present invention provides an amino acid sequence encoding a CH2 region with the P247L modification comprising SEQ ID NO:15. In certain embodiments, the present invention provides a peptide (containing the L251F modification) with the sequence shown in SEQ ID NO: 16. In additional embodiments, the present invention provides a nucleic acid sequence encoding a CH2 region with the L251F modification (e.g. SEQ ID NO:41). In additional embodiments, the present invention provides an amino acid sequence encoding a CH2 region with the L251F modification comprising SEQ ID NO:16.

In some embodiments, the present invention provides a peptide (containing the T256M amino acid modification) comprising the sequence shown in SEQ ID NO:17. In other embodiments, the present invention provides a nucleic acid sequence encoding a CH2 region with the T256M modification (e.g. SEQ ID NO:42). In some embodiments, the present invention provides an amino acid sequence encoding a CH2 region with the T256M modification comprising SEQ ID NO:17. In certain embodiments, the present invention provides a peptide (containing the H268E modification) comprising the sequence shown in SEQ ID NO:20. In additional embodiments, the present invention provides a nucleic acid sequence encoding a CH2 region with the H268E modification (e.g. SEQ ID NO:45). In additional embodiments, the present invention provides an amino acid sequence encoding a CH2 region with the H268E modification comprising SEQ ID NO:20.

In some embodiments, the present invention provides a peptide (containing the D280A amino acid modification) comprising the sequence shown in SEQ ID NO:21. In other embodiments, the present invention provides a nucleic acid sequence encoding a CH2 region with the D280A modification (e.g. SEQ ID NO:46). In some embodiments, the present invention provides an amino acid sequence encoding a CH2 region with the

D280A modification comprising SEQ ID NO:21. In certain embodiments, the present invention provides a peptide (containing the A330K modification) comprising the sequence shown in SEQ ID NO:23. In additional embodiments, the present invention provides a nucleic acid sequence encoding a CH2 region with the A330K modification (e.g. SEQ ID NO:48). In additional embodiments, the present invention provides an amino acid sequence encoding a CH2 region with the A330K modification comprising SEQ ID NO:23.

In some embodiments, the present invention provides a peptide (containing the I332E amino acid modification) comprising the sequence shown in SEQ ID NO:26. In other embodiments, the present invention provides a nucleic acid sequence encoding a CH2 region with the I332E modification (e.g. SEQ ID NO:51). In some embodiments, the present invention provides an amino acid sequence encoding a CH2 region with the I332E modification comprising SEQ ID NO:26. In certain embodiments, the present invention provides a peptide (containing the A339T modification) comprising the sequence showin in SEQ ID NO:29. In additional embodiments, the present invention provides a nucleic acid sequence encoding a CH2 region with the A339T modification (e.g. SEQ ID NO:54). In additional embodiments, the present invention provides an amino acid sequence encoding a CH2 region with the A339T modification comprising SEQ ID NO:29.

In some embodiments, the present invention provides a peptide (containing the A378D amino acid modification) comprising the sequence shown in SEQ ID NO:30. In other embodiments, the present invention provides a nucleic acid sequence encoding a CH2 region with the A378D modification (e.g. SEQ ID NO:55). In some embodiments, the present invention provides an amino acid sequence encoding a CH2 region with the S440Y modification comprising SEQ ID NO:30.

In some embodiments, the present invention provides a peptide (containing the S440Y amino acid modification) comprising the sequence showin in SEQ ID NO:31. In other embodiments, the present invention provides a nucleic acid sequence encoding a CH2 region with the S440Y modification (e.g. SEQ ID NO:56). In some embodiments, the present invention provides an amino acid sequence encoding a CH2 region with the S440Y modification comprising SEQ ID NO:31.

In some embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant comprises at least one amino acid modification at position 247 in the Fc region selected from P247H, P247I and P247L. In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant comprises at least one amino acid modification at position 251 in the Fc region selected from L251F. In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant comprises at least one amino acid modification at position 256 in the Fc region selected from T256M and T256P. In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant comprises at least one amino acid modification at position 268 in the Fc region selected from H268D and H268E. In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant comprises at least one amino acid modification at position 280 in the Fc region selected from D280A and D280K. In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant comprises at least one amino acid modification at position 330 in the Fc region selected from A330K and A330R. In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant comprises at least one amino acid modification at position 332 in the Fc region selected from I332D and I332E. In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant comprises at least one amino acid

modification at position 339 in the Fc region selected from A339T. In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant comprises at least one amino acid modification at position 378 in the Fc region selected from A378D. In other embodiments, the present invention provides compositions comprising a variant (or a nucleic acid sequence encoding the variant) of a parent polypeptide having at least a portion of an Fc region, wherein the variant comprises at least one amino acid modification at position 440 in the Fc region selected from S440Y.

In some embodiments, the present invention provides compositions comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 247 in the Fc region. In other embodiments, the present invention provides methods comprising; a) providing; i) a composition comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 247 in the Fc region, and ii) a subject with one or more symptoms of a disease; and b) administering the composition to the subject under conditions such that at least one of the symptoms is reduced. In particular embodiments the variant comprises an antibody or immunoadhesin, and the subject has symptoms of an antibody or immunoadhesin responsive disease.

In certain embodiments, the variant comprises at least a portion of the Fc region (e.g. 40%, 50%, 60%, 80%, or 90% or more of an Fc region containing the amino acid modification). In some embodiments, the polypeptide variants comprise a CH2 or CH3 region. In further embodiments, the compositions comprise an amino acid sequence comprising SEQ ID NO:13. In some embodiments, the compositions comprise an amino acid sequence comprising SEQ ID NO: 14. In some embodiments, the compositions comprise an amino acid sequence comprising SEQ ID NO: 15. In certain embodiments, the compositions comprise a nucleic acid sequence comprising SEQ ID NO:38 and/or

SEQ ID NO:39 and or SEQ ID NO: 40 or the complement thereof, or sequences that bind to SEQ ID NOs:38, 39, or 40 under conditions of high stringency. In further embodiments, the present invention provides host cells (e.g. CHO cells), and vectors comprising SEQ ID NO:38 and/or SEQ ID NO:39 and or SEQ ID NO: 40. In particular embodiments, the present invention provides a computer readable medium, wherein the computer readable medium encodes a representation of SEQ ID NO:13, 14, 15, 38, 39, or 40.

In certain embodiments, the polypeptide variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide. In some embodiments, the polypeptide variant comprises an antibody or antibody fragment (e.g., polyclonal antibody, monoclonal antibody, chimeric antibody, humanized antibody, or Fc fragment). In some embodiments, the parent polypeptide comprises a human IgG Fc region. In additional embodiments, the parent polypeptide comprises a human IgG1, IgG2, IgG3, or IgG4 Fc region. In other embodiments, the parent polypeptide comprises an amino acid sequence selected from SEQ ID NOS:1-12.

In preferred embodiments, the amino acid modification is P247H, P247I, or P247L. In certain embodiments, the polypeptide variant comprises a second, third, fourth, etc. amino acid modification in the Fc region (see, e.g. Table 1). In some embodiments, the variant is a CHO-expressed polypeptide.

In some embodiments, the present invention provides compositions comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 251 in the Fc region. In other embodiments, the present invention provides methods comprising; a) providing; i) a composition comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 251 in the Fc region, and ii) a subject with one or more symptoms of a disease; and b) administering the composition to the

subject under conditions such that at least one of the symptoms is reduced. In particular embodiments the variant comprises an antibody or immunoadhesin, and the subject has symptoms of an antibody or immunoadhesin responsive disease.

In certain embodiments, the variant comprises at least a portion of the Fc region (e.g. 40%, 50%, 60%, 80%, or 90% or more of an Fc region containing the amino acid modification). In some embodiments, the polypeptide variants comprise a CH2 or CH3 region. In further embodiments, the compositions comprise an amino acid sequence comprising SEQ ID NO:16. In certain embodiments, the compositions comprise a nucleic acid sequence comprising SEQ ID NO:41 or the complement thereof, or sequences that bind to SEQ ID NO:41 and under conditions of high stringency. In further embodiments, the present invention provides host cells (e.g. CHO cells), and vectors comprising SEQ ID NO:41. In particular embodiments, the present invention provides a computer readable medium, wherein the computer readable medium encodes a representation of SEQ ID NO:16 or 41.

In certain embodiments, the polypeptide variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide. In some embodiments, the polypeptide variant comprises an antibody or antibody fragment (e.g., polyclonal antibody, monoclonal antibody, chimeric antibody, humanized antibody, or Fc fragment). In some embodiments, the parent polypeptide comprises a human IgG Fc region. In additional embodiments, the parent polypeptide comprises a human IgG1, IgG2, IgG3, or IgG4 Fc region. In other embodiments, the parent polypeptide comprises an amino acid sequence selected from SEQ ID NOs: 1-12.

In preferred embodiments, the amino acid modification is L251F. In certain embodiments, the polypeptide variant comprises a second, third, fourth, etc. amino acid modification in the Fc region (see, e.g. Table 1). In some embodiments, the variant is a CHO-expressed polypeptide.

In some embodiments, the present invention provides compositions comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one

amino acid modification at position 256 in the Fc region. In other embodiments, the present invention provides methods comprising; a) providing; i) a composition comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 256 in the Fc region, and ii) a subject with one or more symptoms of a disease; and b) administering the composition to the subject under conditions such that at least one of the symptoms is reduced. In particular embodiments the variant comprises an antibody or immunoadhesin, and the subject has symptoms of an antibody or immunoadhesin responsive disease.

In certain embodiments, the variant comprises at least a portion of the Fc region (e.g. 40%, 50%, 60%, 80%, or 90% or more of an Fc region containing the amino acid modification). In some embodiments, the polypeptide variants comprise a CH2 or CH3 region. In further embodiments, the compositions comprise an amino acid sequence comprising SEQ ID NO:17. In some embodiments, the compositions comprise an amino acid sequence comprising SEQ ID NO:18. In certain embodiments, the compositions comprise a nucleic acid sequence comprising SEQ ID NO:42 and/or SEQ ID NO:43, or the complement thereof, or sequences that bind to SEQ ID NOs:42 or 43 under conditions of high stringency. In further embodiments, the present invention provides host cells (e.g. CHO cells), and vectors comprising SEQ ID NO:42 and/or SEQ ID NO:43. In particular embodiments, the present invention provides a computer readable medium, wherein the computer readable medium encodes a representation of SEQ ID NO:17, 18, 42, or 43.

In certain embodiments, the polypeptide variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide. In some embodiments, the polypeptide variant comprises an antibody or antibody fragment (e.g., polyclonal antibody, monoclonal antibody, chimeric antibody, humanized antibody, or Fc fragment). In some embodiments, the parent polypeptide comprises a human IgG Fc region. In additional embodiments, the parent polypeptide comprises a human IgG1, IgG2, IgG3, or IgG4 Fc region. In other embodiments, the parent polypeptide comprises an amino acid sequence selected from SEQ ID NOs:1-12.

In preferred embodiments, the amino acid modification is T256M or T256P. In certain embodiments, the polypeptide variant comprises a second, third, fourth, etc. amino acid modification in the Fc region (see, e.g. Table 1). In some embodiments, the variant is a CHO-expressed polypeptide.

In some embodiments, the present invention provides compositions comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 268 in the Fc region. In other embodiments, the present invention provides methods comprising; a) providing; i) a composition comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 268 in the Fc region, and ii) a subject with one or more symptoms of a disease; and b) administering the composition to the subject under conditions such that at least one of the symptoms is reduced. In particular embodiments the variant comprises an antibody or immunoadhesin, and the subject has symptoms of an antibody or immunoadhesin responsive disease.

In certain embodiments, the variant comprises at least a portion of the Fc region (e.g. 40%, 50%, 60%, 80%, or 90% or more of an Fc region containing the amino acid modification). In some embodiments, the polypeptide variants comprise a CH2 or CH3 region. In further embodiments, the compositions comprise an amino acid sequence comprising SEQ ID NO:19. In some embodiments, the compositions comprise an amino acid sequence comprising SEQ ID NO:20. In certain embodiments, the compositions comprise a nucleic acid sequence comprising SEQ ID NO:44 and/or SEQ ID NO:45, or the complement thereof, or sequences that bind to SEQ ID NOs:44 or 45 under conditions of high stringency. In further embodiments, the present invention provides host cells (e.g. CHO cells), and vectors comprising SEQ ID NO:44 and/or SEQ ID NO:45. In particular embodiments, the present invention provides a computer readable medium, wherein the computer readable medium encodes a representation of SEQ ID NO:19, 20, 44, or 45.

In some embodiments, the present invention provides compositions comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells less effectively than the parent polypeptide and comprises at least one amino acid modification at position 332 in the Fc region. In particular embodiments, the at least one amino acid modification is I332K or I332R.

In certain embodiments, the polypeptide variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide. In some embodiments, the polypeptide variant comprises an antibody or antibody fragment (e.g., polyclonal antibody, monoclonal antibody, chimeric antibody, humanized antibody, or Fc fragment). In some embodiments, the parent polypeptide comprises a human IgG Fc region. In additional embodiments, the parent polypeptide comprises a human IgG1, IgG2, IgG3, or IgG4 Fc region. In other embodiments, the parent polypeptide comprises an amino acid sequence selected from SEQ ID NOs:1-12.

In preferred embodiments, the amino acid modification is H268D or H268E. In certain embodiments, the polypeptide variant comprises a second, third, fourth, etc. amino acid modification in the Fc region (see, e.g. Table 1). In some embodiments, the variant is a CHO-expressed polypeptide.

In some embodiments, the present invention provides compositions comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 280 in the Fc region. In other embodiments, the present invention provides methods comprising; a) providing; i) a composition comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 280 in the Fc region, and ii) a subject with one or more symptoms of a disease; and b) administering the composition to the subject under conditions such that at least one of the symptoms is reduced. In particular

embodiments the variant comprises an antibody or immunoadhesin, and the subject has symptoms of an antibody or immunoadhesin responsive disease.

In certain embodiments, the variant comprises at least a portion of the Fc region (e.g. 40%, 50%, 60%, 80%, or 90% or more of an Fc region containing the amino acid modification). In some embodiments, the polypeptide variants comprise a CH2 or CH3 region. In further embodiments, the compositions comprise an amino acid sequence comprising SEQ ID NO:21. In some embodiments, the compositions comprise an amino acid sequence comprising SEQ ID NO:22. In certain embodiments, the compositions comprise a nucleic acid sequence comprising SEQ ID NO:46 and/or SEQ ID NO:47, or the complement thereof, or sequences that bind to SEQ ID NOs:46 and 47 under conditions of high stringency. In further embodiments, the present invention provides host cells (e.g. CHO cells), and vectors comprising SEQ ID NO:46 and/or SEQ ID NO:47. In particular embodiments, the present invention provides a computer readable medium, wherein the computer readable medium encodes a representation of SEQ ID NO:21, 22, 46 and 47.

In certain embodiments, the polypeptide variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide. In some embodiments, the polypeptide variant comprises an antibody or antibody fragment (e.g., polyclonal antibody, monoclonal antibody, chimeric antibody, humanized antibody, or Fc fragment). In some embodiments, the parent polypeptide comprises a human IgG Fc region. In additional embodiments, the parent polypeptide comprises a human IgG1, IgG2, IgG3, or IgG4 Fc region. In other embodiments, the parent polypeptide comprises an amino acid sequence selected from SEQ ID NOs:1-12.

In preferred embodiments, the amino acid modification is D280A or D280K. In certain embodiments, the polypeptide variant comprises a second, third, fourth, etc. amino acid modification in the Fc region (see, e.g. Table 1). In some embodiments, the variant is a CHO-expressed polypeptide.

In some embodiments, the present invention provides compositions comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence

of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 330 in the Fc region. In other embodiments, the present invention provides methods comprising; a) providing; i) a composition comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 330 in the Fc region, and ii) a subject with one or more symptoms of a disease; and b) administering the composition to the subject under conditions such that at least one of the symptoms is reduced. In particular embodiments the variant comprises an antibody or immunoadhesin, and the subject has symptoms of an antibody or immunoadhesin responsive disease.

In certain embodiments, the variant comprises at least a portion of the Fc region (e.g. 40%, 50%, 60%, 80%, or 90% or more of an Fc region containing the amino acid modification). In some embodiments, the polypeptide variants comprise a CH2 or CH3 region. In further embodiments, the compositions comprise an amino acid sequence comprising SEQ ID NO:23. In some embodiments, the compositions comprise an amino acid sequence comprising SEQ ID NO:24. In certain embodiments, the compositions comprise a nucleic acid sequence comprising SEQ ID NO:48 and/or SEQ ID NO:49, or the complement thereof, or sequences that bind to SEQ ID NOs:48 or 49 under conditions of high stringency. In further embodiments, the present invention provides host cells (e.g. CHO cells), and vectors comprising SEQ ID NO:48 and/or SEQ ID NO:49. In particular embodiments, the present invention provides a computer readable medium, wherein the computer readable medium encodes a representation of SEQ ID NOs:23, 24, 48 and 49.

In certain embodiments, the polypeptide variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide. In some embodiments, the polypeptide variant comprises an antibody or antibody fragment (e.g., polyclonal antibody, monoclonal antibody, chimeric antibody, humanized antibody, or Fc fragment). In some embodiments, the parent polypeptide comprises a human IgG Fc region. In additional embodiments, the parent polypeptide comprises a human IgG1, IgG2, IgG3, or IgG4 Fc region. In other

embodiments, the parent polypeptide comprises an amino acid sequence selected from SEQ ID NOs:1-12.

In preferred embodiments, the amino acid modification is A330K or A330R. In certain embodiments, the polypeptide variant comprises a second, third, fourth, etc. amino acid modification in the Fc region (see, e.g. Table 1). In some embodiments, the variant is a CHO-expressed polypeptide.

In some embodiments, the present invention provides compositions comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 332 in the Fc region. In other embodiments, the present invention provides methods comprising; a) providing; i) a composition comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 332 in the Fc region, and ii) a subject with one or more symptoms of a disease; and b) administering the composition to the subject under conditions such that at least one of the symptoms is reduced. In particular embodiments the variant comprises an antibody or immunoadhesin, and the subject has symptoms of an antibody or immunoadhesin responsive disease.

In certain embodiments, the variant comprises at least a portion of the Fc region (e.g. 40%, 50%, 60%, 80%, or 90% or more of an Fc region containing the amino acid modification). In some embodiments, the polypeptide variants comprise a CH2 or CH3 region. In further embodiments, the compositions comprise an amino acid sequence comprising SEQ ID NO:25. In some embodiments, the compositions comprise an amino acid sequence comprising SEQ ID NO:26. In certain embodiments, the compositions comprise a nucleic acid sequence comprising SEQ ID NO:50 and/or SEQ ID NO:51, or the complement thereof, or sequences that bind to SEQ ID NOs:50 or 51 under conditions of high stringency. In further embodiments, the present invention provides host cells (e.g. CHO cells), and vectors comprising SEQ ID NO:50 and/or SEQ ID NO:51. In particular

embodiments, the present invention provides a computer readable medium, wherein the computer readable medium encodes a representation of SEQ ID NO:25, 26, 50, and 51.

In certain embodiments, the polypeptide variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide. In some embodiments, the polypeptide variant comprises an antibody or antibody fragment (e.g., polyclonal antibody, monoclonal antibody, chimeric antibody, humanized antibody, or Fc fragment). In some embodiments, the parent polypeptide comprises a human IgG Fc region. In additional embodiments, the parent polypeptide comprises a human IgG1, IgG2, IgG3, or IgG4 Fc region. In other embodiments, the parent polypeptide comprises an amino acid sequence selected from SEQ ID NOs:1-12.

In preferred embodiments, the amino acid modification is I332D or I332E. In certain embodiments, the polypeptide variant comprises a second, third, fourth, etc. amino acid modification in the Fc region (see, e.g. Table 1). In some embodiments, the variant is a CHO-expressed polypeptide.

In some embodiments, the present invention provides compositions comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 339 in the Fc region. In other embodiments, the present invention provides methods comprising; a) providing; i) a composition comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 339 in the Fc region, and ii) a subject with one or more symptoms of a disease; and b) administering the composition to the subject under conditions such that at least one of the symptoms is reduced. In particular embodiments the variant comprises an antibody or immunoadhesin, and the subject has symptoms of an antibody or immunoadhesin responsive disease.

In certain embodiments, the variant comprises at least a portion of the Fc region (e.g. 40%, 50%, 60%, 80%, or 90% or more of an Fc region containing the amino acid

modification). In some embodiments, the polypeptide variants comprise a CH2 or CH3 region. In further embodiments, the compositions comprise an amino acid sequence comprising SEQ ID NO:29. In certain embodiments, the compositions comprise a nucleic acid sequence comprising SEQ ID NO:54, or the complement thereof, or sequences that bind to SEQ ID NO:54 under conditions of high stringency. In further embodiments, the present invention provides host cells (e.g. CHO cells), and vectors comprising SEQ ID NO:54. In particular embodiments, the present invention provides a computer readable medium, wherein the computer readable medium encodes a representation of SEQ ID NOs:29 or 54.

In certain embodiments, the polypeptide variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide. In some embodiments, the polypeptide variant comprises an antibody or antibody fragment (e.g., polyclonal antibody, monoclonal antibody, chimeric antibody, humanized antibody, or Fc fragment). In some embodiments, the parent polypeptide comprises a human IgG Fc region. In additional embodiments, the parent polypeptide comprises a human IgG1, IgG2, IgG3, or IgG4 Fc region. In other embodiments, the parent polypeptide comprises an amino acid sequence selected from SEQ ID NOs:1-12.

In preferred embodiments, the amino acid modification is A339T. In certain embodiments, the polypeptide variant comprises a second third, fourth, etc. amino acid modification in the Fc region (see, e.g. Table 1). In some embodiments, the variant is a CHO-expressed polypeptide.

In some embodiments, the present invention provides compositions comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates the depletion of target cells in a whole blood assay more effectively than the parent polypeptide and comprises at least one amino acid modification at position 378 in the Fc region. In other embodiments, the present invention provides methods comprising; a) providing; i) a composition comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates the depletion of target cells in a whole blood assay more effectively than the parent polypeptide and comprises at least one amino acid modification at position 378 in the Fc region, and ii) a

subject with one or more symptoms of a disease; and b) administering the composition to the subject under conditions such that at least one of the symptoms is reduced. In particular embodiments the variant comprises an antibody or immunoadhesin, and the subject has symptoms of an antibody or immunoadhesin responsive disease.

In certain embodiments, the variant comprises at least a portion of the Fc region (e.g. 40%, 50%, 60%, 80%, or 90% or more of an Fc region containing the amino acid modification). In some embodiments, the polypeptide variants comprise a CH2 or CH3 region. In further embodiments, the compositions comprise an amino acid sequence comprising SEQ ID NO:30. In certain embodiments, the compositions comprise a nucleic acid sequence comprising SEQ ID NO:55, or the complement thereof, or sequences that bind to SEQ ID NO:55 under conditions of high stringency. In further embodiments, the present invention provides host cells (e.g. CHO cells), and vectors comprising SEQ ID NO:55. In particular embodiments, the present invention provides a computer readable medium, wherein the computer readable medium encodes a representation of SEQ ID NOs:30 or 55.

In certain embodiments, the polypeptide variant mediates mediates the depletion of target cells in a whole blood assay more effectively than the parent polypeptide. In some embodiments, the polypeptide variant comprises an antibody or antibody fragment (e.g., polyclonal antibody, monoclonal antibody, chimeric antibody, humanized antibody, or Fc fragment). In some embodiments, the parent polypeptide comprises a human IgG Fc region. In additional embodiments, the parent polypeptide comprises a human IgG1, IgG2, IgG3, or IgG4 Fc region. In other embodiments, the parent polypeptide comprises an amino acid sequence selected from SEQ ID NOs:1-12. In preferred embodiments, the amino acid modification is A378D. In certain embodiments, the polypeptide variant comprises a second, third, fourth, etc. amino acid modification in the Fc region (see, e.g. Table 1). In some embodiments, the variant is a CHO-expressed polypeptide.

In some embodiments, the present invention provides compositions comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 440 in the Fc region. In other embodiments, the

present invention provides methods comprising; a) providing; i) a composition comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide and comprises at least one amino acid modification at position 440 in the Fc region, and ii) a subject with one or more symptoms of a disease; and b) administering the composition to the subject under conditions such that at least one of the symptoms is reduced. In particular embodiments the variant comprises an antibody or immunoadhesin, and the subject has symptoms of an antibody or immunoadhesin responsive disease.

In certain embodiments, the variant comprises at least a portion of the Fc region (e.g. 40%, 50%, 60%, 80%, or 90% or more of an Fc region containing the amino acid modification). In some embodiments, the polypeptide variants comprise a CH2 or CH3 region. In further embodiments, the compositions comprise an amino acid sequence comprising SEQ ID NO:31. In certain embodiments, the compositions comprise a nucleic acid sequence comprising SEQ ID NO:56, or the complement thereof, or sequences that bind to SEQ ID NO:56 under conditions of high stringency. In further embodiments, the present invention provides host cells (e.g. CHO cells), and vectors comprising SEQ ID NO:56. In particular embodiments, the present invention provides a computer readable medium, wherein the computer readable medium encodes a representation of SEQ ID NOs:31 or 56.

In certain embodiments, the polypeptide variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide. In some embodiments, the polypeptide variant comprises an antibody or antibody fragment (e.g., polyclonal antibody, monoclonal antibody, chimeric antibody, humanized antibody, or Fc fragment). In some embodiments, the parent polypeptide comprises a human IgG Fc region. In additional embodiments, the parent polypeptide comprises a human IgG1, IgG2, IgG3, or IgG4 Fc region. In other embodiments, the parent polypeptide comprises an amino acid sequence selected from SEQ ID NOs:1-12.

In preferred embodiments, the amino acid modification is S440Y. In certain embodiments, the polypeptide variant comprises a second, third, fourth, etc. amino acid

modification in the Fc region (see, e.g. Table 1). In some embodiments, the variant is a CHO-expressed polypeptide.

In further embodiments, the compositions comprise a nucleic acid sequence encoding a variant of a parent polypeptide comprising at least a portion of an Fc region, wherein the variant mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively that the parent polypeptide, and comprises at least one amino acid modification at position 247, 251, 256, 268, 280, 330, 332, 339, 378, or 440, or combinations thereof in the Fc region. In some embodiments, the compositions comprise a nucleic acid sequence encoding a variant Fc polypeptide which mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells more effectively than the parent polypeptide, wherein the variant Fc polypeptide comprises an amino acid modification at amino acid position 247, 251, 256, 268, 280, 330, 332, 339, 378, or 440, or combinations thereof.

In further embodiments, the compositions comprise a nucleic acid sequence encoding a variant of a parent polypeptide comprising at least a portion of an Fc region, wherein the variant mediates the depletion of target cells in a whole blood assay more effectively than the parent polypeptide, and comprises at least one amino acid modification at position 247, 251, 256, 268, 280, 330, 332, 339, 378, or 440, or combinations thereof in the Fc region. In some embodiments, the compositions comprise a nucleic acid sequence encoding a variant Fc polypeptide which mediates the depletion of target cells in a whole blood assay more effectively than the parent polypeptide, wherein the variant Fc polypeptide comprises an amino acid modification at amino acid position 247, 251, 256, 268, 280, 330, 332, 339, 378, or 440, or combinations thereof.

In certain embodiments, the variants of the present invention, and the nucleic acid sequences encoding the variants, are provided with at least one other component in a kit. For example, a kit may comprise at least one type of variant, and written instructions for using the variant. The kit may also contain buffers, and other useful reagents.

In some embodiments, the present invention provides methods comprising, a) providing; i) cells expressing target antigen (CD20, for eg.), ii) a composition comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant comprises at least one amino acid modification in the Fc region, and iii) effector

cells (enriched PBMCs from human donor(s), for example), and b) contacting the target cells with the composition and effector cells under conditions such that the variant binds to the target cells (e.g. via a ligand expressed on the cell surface) and c) measuring killing of the target cells (release of LDH or chromium 51, for example).

In other embodiments, the present invention provides methods comprising, a) providing; i) whole blood samples containing cells expressing target antigen (B cells expressing CD20, for e.g.) and ii) a composition comprising a variant of a parent polypeptide having at least a portion of an Fc region, wherein the variant comprises at least one amino acid modification in the Fc region and b) contacting the target cells with the composition under conditions such that the variant binds to the target cells (e.g. via the CD20 ligand expressed on the cell surface) and c) measuring the disappearance of the target cells (Facs analysis with other B cell marker, such as CD19, for example).

In some embodiments, the present invention provides methods of identifying dual-species improved variants, comprising; a) providing; i) target cells, ii) a composition comprising a candidate variant of a parent polypeptide having an Fc region, wherein the candidate variant comprises at least one amino acid modification in the Fc region, and wherein the candidate variant mediates target cell cytotoxicity in the presence of a first species of effector cells more effectively than the parent polypeptide, and iii) second species effector cells, and b) incubating the composition with the target cells under conditions such that the candidate variant binds the target cells thereby generating candidate variant bound target cells, c) mixing the second species effector cells with the candidate variant bound target cells, d) measuring target cell cytotoxicity (e.g. mediated by the candidate variant), e) determining if the candidate variant mediates target cell cytotoxicity in the presence of the second species effector cells more effectively than the parent polypeptide. In some embodiments, the method further comprises screening the parent polypeptide in the same fashion with the second species effector cells. In further embodiments, steps b) and c) are performed simultaneously. In particular embodiments, the method further comprises step f) identifying a candidate variant as a dual-species improved variant that mediates target cell cytotoxicity in the presence of the second species effector cells more effectively than the parent polypeptide. In other embodiments, the method further comprises step f) identifying a candidate variant as a dual-species

improved variant that mediates target cell cytotoxicity in the presence of the second species effector cells about 1.2 times (or about 1.5 times, 5 times, or about 10 times) more effectively than the parent polypeptide (e.g. about 1.2 times more target cell lysis is observed).

In other embodiments, the present invention provides methods of identifying dual-species improved variants, comprising; a) providing; i) target cells, ii) a composition comprising a candidate variant of a parent polypeptide having an Fc region, wherein the candidate variant comprises at least one amino acid modification in the Fc region, iii) first species effector cells, and iv) second species effector cells, and b) incubating the composition with the target cells under conditions such that the candidate variant binds the target cells thereby generating candidate variant bound target cells, c) mixing the first species effector cells with the candidate variant bound target cells, d) measuring target cell cytotoxicity (e.g. mediated by the candidate variant), e) determining that the candidate variant mediates target cell cytotoxicity in the presence of the first species effector cells more effectively than the parent polypeptide, f) mixing the second species effector cells with the candidate variant bound target cells (e.g. as generated in step b), g) measuring target cell cytotoxicity (e.g. mediated by the candidate variant), h) determining if the candidate variant mediates target cell cytotoxicity in the presence of the second species effector cells more effectively than the parent polypeptide.

In particular embodiments, the method further comprises a step to determine the ability of the parent polypeptide to mediate target cell cytotoxicity in the presence of the first species and/or the second species. For example, the methods may further comprise mixing the first or second species effector cells with parent polypeptide bound target cells, and then measuring target cell cytotoxicity (e.g. determining a value such that there is a value to compare the variants against).

In certain embodiments, the method further comprises step g) administering the dual-species improved variant to a test animal, wherein the test animal is a member of the second species. In other embodiments, the method further comprises, prior to step a), a step of screening the candidate variant in an Fc receptor (FcR) binding assay. In certain embodiments, the FcR binding assay is an Fc neonatal receptor (FcRn) binding assay.

In some embodiments, the method further comprises, prior to step a), a step of screening the candidate variant in a CDC assay (See, e.g., section IV below). In some embodiments, the first species of effector cells are human peripheral blood mononuclear cells (PBMCs). In other embodiments, the first species of effector cells are mouse PBMCs or rat PBMCs. In certain embodiments, the second species of effector cells are mouse PBMCs or rat PBMCs. In particular embodiments, the second species of effector cells are human PBMCs.

In some embodiments, the present invention provides methods of identifying dual-species improved variants, comprising; a) providing; i) a composition comprising a candidate variant of a parent polypeptide having an Fc region, wherein the candidate variant comprises at least one amino acid modification in the Fc region, and wherein the candidate variant mediates CDC more effectively than the parent polypeptide, and iii) a second species source of complement, and b) incubating the composition with the second species of complement; and c) determining if the candidate variant mediates CDC more effectively than the parent polypeptide. In particular embodiments, the method further comprises step d) identifying a candidate variant as a dual-species improved variant.

In some embodiments, the target cells are human cells (e.g. over-expressing one or more of the following tumor-associated antigens: CD20, CD22, CD33, CD40, CD63, EGF receptor, her-2 receptor, prostate-specific membrane antigen, Lewis Y carbohydrate, GD_2 and GD_3 gangliosides, lamp-1, CO-029, L6, and ephA2). In certain embodiments, the variant comprises an antibody, or portion thereof, specific for the target cells. In other embodiments, the candidate variant mediates target cell cytoxicity in the presence of the first species of effector cells about 1.2 times more effectively than the parent polypeptide. In some embodiments, step e) comprises performing a control reaction with the parent polypeptide. In additional embodiments, the measuring comprises quantitating target cell death or target cell lysis. In other embodiments, the target cells infected with viruses (e.g. HIV, CMV, hepatitis B, or RSV, for example) or microbial organisms (e.g. Staphylococcus, Streptococcus, Pseudomonas, etc). In certain embodiments, the target cells are microbial organisms (e.g. Staphylococcus, Streptococcus, Pseudomonas, etc). In some embodiments, the target cells are replaced instead with viruses (e.g. HIV, CMV, hepatitis B, or RSV).

In some embodiments, the present invention provides compositions comprising a CD20 binding molecule, or a nucleic acid sequence encoding a CD20 binding molecule, wherein the CD20 binding molecule comprises a light chain variable region, and wherein the light chain variable region comprises the amino acid sequence shown in SEQ ID NO:57. In other embodiments, the present invention provides compositions composition comprising a CD20 binding mólecule, or a nucleic acid sequence encoding a CD20 binding molecule, wherein the CD20 binding molecule comprises a heavy chain variable region, and wherein the heavy chain variable region comprises the amino acid sequence shown in SEQ ID NO:58. In certain embodiments, the present invention provides compositions comprising a CD20 binding molecule, or a nucleic acid sequence encoding a CD20 binding molecule (See, e.g. Figure 16), wherein the CD20 binding molecule comprises a light chain variable region and a heavy chain variable region, wherein the light chain variable region comprises the amino acid sequence shown in SEQ ID NO:57 (See Figure 15), and wherein the heavy chain variable regin comprises the amino acid sequence shown in SEQ ID NO:58 (See Figure 15).

In some embodiments, the present invention provides compositions comprising a polypeptide, wherein the polypeptide comprises; i) an unmodified human framework (e.g. no alterations have been made to a naturally occurring human framework), and ii) a variant Fc region. In certain embodiments, the unmodified human framework is a human germline framework. In other embodiments, the present invention provides compositions comprising a polypeptide, wherein the polypeptide comprises: i) at least one randomized CDR sequence and ii) a variant Fc region. In further embodiments, the present invention provides compositions comprising a polypeptide, wherein the polypeptide comprises; i) an unmodified human framework (e.g. human germline framework), ii) at least one randomized CDR sequence, and iii) a variant Fc region.

DESCRIPTION OF THE FIGURES

Figure 1 shows a schematic representation of an IgG molecule with the various regions and sections labeled.

Figure 2 shows an alignment of various parental Fc amino acid sequences, including human IgG1 (with non-a (SEQ ID NO:1) and a allotypes shown), human IgG2

(SEQ ID NO:2), human IgG3 (SEQ ID NO:3), human IgG4 (SEQ ID NO:4), murine IgG1 (SEQ ID NO:5), murine IgG2A (SEQ ID NO:6), murine IgG2B (SEQ ID NO:7), and murine IgG3 (SEQ ID NO:8).

Figure 3 shows various amino acid sequences, including the CH2 region (SEQ ID NO:9), and CH3 region (SEQ ID NO:10) of human IgG1, as well as an f allotype (SEQ ID NO:11) and a, z allotype (SEQ ID NO:12) sequences of human IgG1 that include the CH1, hinge, CH2 and CH3 regions.

Figure 4 shows the altered amino acid sequences of the variants in the context of local surrounding sequences.

Figure 5 shows nucleotide sequences encoding parental polypeptides.

Figure 6 shows the altered nucleic acid sequences of the variants in the context of local surrounding nucleic acid sequences.

Figure 7A shows the results of an ADCC assay comparing the wild type Fc region with A330K, A330R, I332E and the combination of mutations at both positions 330 and 332.

Figure 7B shows the results of an ADCC assay comparing the Fc region amino acid variants I332D and I332E to the Fc region glycan modification variant 33 (GnTIII) and the effect of amino acid plus glycan modification 33 (I332E + GnTIII) on ADCC activity.

Figure 8 shows representative ADCC data obtained using purified variant IgG.

Figure 9 shows ADCC activities of variable region variants with low affinity (6F1) and relatively high affinity (33 and 5) to CD20 antigen against Wil-2 (A) or SKW6.4 (B) target cell lines.

Figure 10 shows the titration curves of FcγRI binding to Fc-region variant IgG.

Figure 11A, B, and C show representative CDC data obtained from titration of Fc-region variants using human complement and Ramos target cells.

Figure 12 shows representative B cell depletion with AME-133 (variable region 33 with the I332E Fc variant sequence), Rituxan, and non-specific IgG using a whole blood sample containing the VV (high affinity) genotype of the FcγRIIIa receptor.

Figure 13 shows representative B cell depletion with AME-133 (variable region 33 with the I332E Fc variant sequence), Rituxan, and non-specific IgG using a whole blood sample containing the FF (low affinity) genotype of the FcγRIIIa receptor.

Figure 14 shows representative B cell depletion with Rituxan, non-specific IgG and with variants A378D and T256P, I332E using a whole blood sample.

Figure 15 shows the amino acid sequence of the light (SEQ ID NO:57) and the heavy (SEQ ID NO:58) chains of AME-133 (variable region 33 with the I332E Fc variant sequence).

Figure 16 shows the nucleotide sequence of the light (SEQ ID NO:59) and the heavy (SEQ ID NO:60) chains of AME-133 (variable region 33 with the I332E Fc variant sequence).

DEFINITIONS

To facilitate an understanding of the invention, a number of terms are defined below.

As used herein, the terms "subject" and "patient" refer to any animal, such as a mammal like a dog, cat, bird, livestock, and preferably a human (e.g. a human with a disease).

As used herein, the terms "nucleic acid molecule encoding," "DNA sequence encoding," and "DNA encoding" refer to the order or sequence of deoxyribonucleotides along a strand of deoxyribonucleic acid. The order of these deoxyribonucleotides determines the order of amino acids along the polypeptide (protein) chain. The DNA sequence thus codes for the amino acid sequence.

DNA molecules are said to have "5' ends" and "3' ends" because mononucleotides are reacted to make oligonucleotides or polynucleotides in a manner such that the 5' phosphate of one mononucleotide pentose ring is attached to the 3' oxygen of its neighbor in one direction via a phosphodiester linkage. Therefore, an end of an oligonucleotides or polynucleotide, referred to as the "5' end" if its 5' phosphate is not linked to the 3' oxygen of a mononucleotide pentose ring and as the "3' end" if its 3' oxygen is not linked to a 5' phosphate of a subsequent mononucleotide pentose ring. As used herein, a nucleic acid sequence, even if internal to a larger oligonucleotide or polynucleotide, also may be said

to have 5' and 3' ends. In either a linear or circular DNA molecule, discrete elements are referred to as being "upstream" or 5' of the "downstream" or 3' elements. This terminology reflects the fact that transcription proceeds in a 5' to 3' fashion along the DNA strand. The promoter and enhancer elements that direct transcription of a linked gene are generally located 5' or upstream of the coding region. However, enhancer elements can exert their effect even when located 3' of the promoter element and the coding region. Transcription termination and polyadenylation signals are located 3' or downstream of the coding region.

As used herein, the term "codon" or "triplet" refers to a tuplet of three adjacent nucleotide monomers which specify one of the twenty naturally occurring amino acids found in polypeptide biosynthesis. The term also includes nonsense codons which do not specify any amino acid.

As used herein, the terms "an oligonucleotide having a nucleotide sequence encoding a polypeptide", "polynucleotide having a nucleotide sequence encoding a polypeptide", and "nucleic acid sequence encoding a polypeptide" means a nucleic acid sequence comprising the coding region of a particular polypeptide. The coding region may be present in a cDNA, genomic DNA, or RNA form. When present in a DNA form, the oligonucleotide or polynucleotide may be single-stranded (*i.e.*, the sense strand) or double-stranded. Suitable control elements such as enhancers/promoters, splice junctions, polyadenylation signals, etc. may be placed in close proximity to the coding region of the gene if needed to permit proper initiation of transcription and/or correct processing of the primary RNA transcript. Alternatively, the coding region utilized in the expression vectors of the present invention may contain endogenous enhancers/promoters, splice junctions, intervening sequences, polyadenylation signals, *etc.* or a combination of both endogenous and exogenous control elements.

As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (*i.e.*, a sequence of nucleotides) related by the base-pairing rules. For example, for the sequence "A-G-T," is complementary to the sequence "T-C-A." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between

nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

As used herein, the term "the complement of" a given sequence is used in reference to the sequence that is completely complementary to the sequence over its entire length. For example, the sequence A-G-T-A is "the complement" of the sequence T-C-A-T.

The term "homology" (when in reference to nucleic acid sequences) refers to a degree of complementarity. There may be partial homology or complete homology (*i.e.*, identity). A partially complementary sequence is one that at least partially inhibits a completely complementary sequence from hybridizing to a target nucleic acid and is referred to using the functional term "substantially homologous." The term "inhibition of binding," when used in reference to nucleic acid binding, refers to inhibition of binding caused by competition of homologous sequences for binding to a target sequence. The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (*i.e.*, the hybridization) of a completely homologous sequence to a target under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (*i.e.*, selective) interaction. The absence of non-specific binding may be tested by the use of a second target that lacks even a partial degree of complementarity (*e.g.*, less than about 30% identity); in the absence of non-specific binding the probe will not hybridize to the second non-complementary target.

The art knows well that numerous equivalent conditions may be employed to comprise low stringency conditions; factors such as the length and nature (DNA, RNA, base composition) of the probe and nature of the target (DNA, RNA, base composition, present in solution or immobilized, etc.) and the concentration of the salts and other components (*e.g.*, the presence or absence of formamide, dextran sulfate, polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions

of low stringency hybridization different from, but equivalent to, the above listed conditions. In addition, the art knows conditions that promote hybridization under conditions of high stringency (*e.g.*, increasing the temperature of the hybridization and/or wash steps, the use of formamide in the hybridization solution, etc.).

A nucleic acid sequence (e.g. encoding a variant Fc region or portion thereof) may produce multiple RNA species that are generated by differential splicing of the primary RNA transcript. cDNAs that are splice variants of the same gene will contain regions of sequence identity or complete homology (representing the presence of the same exon or portion of the same exon on both cDNAs) and regions of complete non-identity (for example, representing the presence of exon "A" on cDNA 1 wherein cDNA 2 contains exon "B" instead). Because the two cDNAs contain regions of sequence identity they will both hybridize to a probe derived from the entire gene or portions of the gene containing sequences found on both cDNAs; the two splice variants are therefore substantially homologous to such a probe and to each other.

When used in reference to a single-stranded nucleic acid sequence, the term "substantially homologous" refers to any probe that can hybridize (*i.e.*, it is the complement of) the single-stranded nucleic acid sequence under conditions of low stringency.

As used herein, the term "hybridization" is used in reference to the pairing of complementary nucleic acids. Hybridization and the strength of hybridization (*i.e.*, the strength of the association between the nucleic acids) is impacted by such factors as the degree of complementary between the nucleic acids, stringency of the conditions involved, the T_m of the formed hybrid, and the G:C ratio within the nucleic acids.

As used herein, the term "T_m" is used in reference to the "melting temperature." The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. The equation for calculating the T_m of nucleic acids is well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation: $T_m = 81.5 + 0.41(\% G + C)$, when a nucleic acid is in aqueous solution at 1 M NaCl (*See e.g.*, Anderson and Young, Quantitative Filter Hybridization, *in Nucleic Acid Hybridization*

[1985]). Other references include more sophisticated computations that take structural as well as sequence characteristics into account for the calculation of T_m, and in some cases the T_m may be determined empirically by beginning with the calculated T_m and testing small increases or decreases of temperature and examining the effect on the population of nucleic acid molecules.

As used herein the term "stringency" is used in reference to the conditions of temperature, ionic strength, and the presence of other compounds such as organic solvents, under which nucleic acid hybridizations are conducted. Those skilled in the art will recognize that "stringency" conditions may be altered by varying the parameters just described either individually or in concert. With "high stringency" conditions, nucleic acid base pairing will occur only between nucleic acid fragments that have a high frequency of complementary base sequences (*e.g.*, hybridization under "high stringency" conditions may occur between homologs with about 85-100% identity, preferably about 70-100% identity). With medium stringency conditions, nucleic acid base pairing will occur between nucleic acids with an intermediate frequency of complementary base sequences (*e.g.*, hybridization under "medium stringency" conditions may occur between homologs with about 50-70% identity). Thus, conditions of "weak" or "low" stringency are often required with nucleic acids that are derived from organisms that are genetically diverse, as the frequency of complementary sequences is usually less.

"High stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42 °C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH_2PO_4 H_2O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 0.1X SSPE, 1.0% SDS at 42 C when a probe of about 500 nucleotides in length is employed.

"Medium stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH_2PO_4 H_2O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml

denatured salmon sperm DNA followed by washing in a solution comprising 1.0X SSPE, 1.0% SDS at 42 C when a probe of about 500 nucleotides in length is employed.

"Low stringency conditions" comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH_2PO_4 H_2O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.1% SDS, 5X Denhardt's reagent [50X Denhardt's contains per 500 ml: 5 g Ficoll (Type 400, Pharmacia), 5 g BSA (Fraction V; Sigma)] and 100 g/ml denatured salmon sperm DNA followed by washing in a solution comprising 5X SSPE, 0.1% SDS at 42 C when a probe of about 500 nucleotides in length is employed.

The following terms are used to describe the sequence relationships between two or more polynucleotides: "reference sequence", "sequence identity", and "percentage of sequence identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA sequence given in a sequence listing or may comprise a complete gene sequence. Generally, a reference sequence is at least 20 nucleotides in length, frequently at least 25 nucleotides in length, and often at least 50 nucleotides in length (*e.g.*any one of SEQ ID NOs: 32-37 may be used as a reference sequence). Since two polynucleotides may each (1) comprise a sequence (*i.e.*, a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) may further comprise a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window", as used herein, refers to a conceptual segment of at least 20 contiguous nucleotide positions wherein a polynucleotide sequence may be compared to a reference sequence of at least 20 contiguous nucleotides and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local

homology algorithm of Smith and Waterman [Smith and Waterman, *Adv. Appl. Math.* 2: 482 (1981)] by the homology alignment algorithm of Needleman and Wunsch [Needleman and Wunsch, *J. Mol. Biol.* 48:443 (1970)], by the search for similarity method of Pearson and Lipman [Pearson and Lipman, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:2444 (1988)], by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by inspection, and the best alignment (*i.e.*, resulting in the highest percentage of homology over the comparison window) generated by the various methods is selected. The term "sequence identity" means that two polynucleotide sequences are identical (*i.e.*, on a nucleotide-by-nucleotide basis) over the window of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The window of comparison, as used in the present application, is the entire length of the recited reference sequence (*i.e.* if SEQ ID NO:33 is recited as the reference sequence, percentage of sequence identity is compared over the entire length of SEQ ID NO:33).

As used herein, the term "probe" refers to an oligonucleotide (*i.e.*, a sequence of nucleotides), whether occurring naturally as in a purified restriction digest or produced synthetically, recombinantly or by PCR amplification, that is capable of hybridizing to another oligonucleotide of interest. A probe may be single-stranded or double-stranded. Probes are useful in the detection, identification and isolation of particular gene sequences. It is contemplated that any probe used in the present invention may be labeled with any "reporter molecule," so that is detectable in any detection system, including, but not limited to enzyme (*e.g.*, ELISA, as well as enzyme-based histochemical assays), fluorescent, radioactive, and luminescent systems. It is not intended that the present invention be limited to any particular detection system or label.

As used herein, the term "polymerase chain reaction" ("PCR") refers to the method described in U.S. Patent Nos. 4,683,195, 4,683,202, and 4,965,188, hereby incorporated by reference, that describe a method for increasing the concentration of a segment of a target sequence in a mixture of genomic DNA without cloning or purification. This process for amplifying the target sequence consists of introducing a large excess of two oligonucleotide primers to the DNA mixture containing the desired target sequence, followed by a precise sequence of thermal cycling in the presence of a DNA polymerase. The two primers are complementary to their respective strands of the double stranded target sequence. To effect amplification, the mixture is denatured and the primers then annealed to their complementary sequences within the target molecule. Following annealing, the primers are extended with a polymerase so as to form a new pair of complementary strands. The steps of denaturation, primer annealing, and polymerase extension can be repeated many times (*i.e.*, denaturation, annealing and extension constitute one "cycle"; there can be numerous "cycles") to obtain a high concentration of an amplified segment of the desired target sequence. The length of the amplified segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to as the "polymerase chain reaction" (hereinafter "PCR"). Because the desired amplified segments of the target sequence become the predominant sequences (in terms of concentration) in the mixture, they are said to be "PCR amplified."

With PCR, it is possible to amplify a single copy of a specific target sequence in genomic DNA to a level detectable by several different methodologies (*e.g.*, hybridization with a labeled probe; incorporation of biotinylated primers followed by avidin-enzyme conjugate detection; incorporation of ^{32}P-labeled deoxynucleotide triphosphates, such as dCTP or dATP, into the amplified segment). In addition to genomic DNA, any oligonucleotide or polynucleotide sequence can be amplified with the appropriate set of primer molecules. In particular, the amplified segments created by the PCR process itself are, themselves, efficient templates for subsequent PCR amplifications.

The term "isolated" when used in relation to a nucleic acid, as in "an isolated oligonucleotide" or "isolated polynucleotide" refers to a nucleic acid sequence that is identified and separated from at least one contaminant nucleic acid with which it is ordinarily associated in its natural source. Isolated nucleic acid is present in a form or setting that is different from that in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule includes a nucleic acid molecule contained in cells that ordinarily express the polypeptide where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells. The isolated nucleic acid, oligonucleotide, or polynucleotide may be present in single-stranded or double-stranded form. When an isolated nucleic acid, oligonucleotide or polynucleotide is to be utilized to express a protein, the oligonucleotide or polynucleotide will contain at a minimum the sense or coding strand (*i.e.*, the oligonucleotide or polynucleotide may single-stranded), but may contain both the sense and anti-sense strands (*i.e.*, the oligonucleotide or polynucleotide may be double-stranded).

As used herein the terms "portion" when used in reference to a nucleotide sequence (as in "a portion of a given nucleotide sequence") refers to fragments of that sequence. The fragments may range in size from ten nucleotides to the entire nucleotide sequence minus one nucleotide (e.g., 10 nucleotides, 20, 30, 40, 50, 100, 200, etc.).

As used herein the term "portion" when in reference to an amino acid sequence (as in "a portion of a given amino acid sequence") refers to fragments of that sequence. The fragments may range in size from six amino acids to the entire amino acid sequence minus one amino acid (e.g., 6 amino acids, 10, 20, 30, 40, 75, 200, etc.).

As used herein, the term "purified" or "to purify" refers to the removal of contaminants from a sample. For example, antigen specific antibodies may be purified by removal of contaminating non-immunoglobulin proteins; they are also purified by the removal of immunoglobulin that does not bind to the same antigen. The removal of non-immunoglobulin proteins and/or the removal of immunoglobulins that do not bind a particular antigen results in an increase in the percent of antigen specific immunoglobulins in the sample. In another example, recombinant antigen specific polypeptides are expressed in bacterial host cells and the polypeptides are purified by the

removal of host cell proteins; the percent of recombinant antigen specific polypeptides is thereby increased in the sample.

The term "recombinant DNA molecule" as used herein refers to a DNA molecule that is comprised of segments of DNA joined together by means of molecular biological techniques.

The term "recombinant protein" or "recombinant polypeptide" as used herein refers to a protein molecule that is expressed from a recombinant DNA molecule.

The term "native protein" as used herein to indicate that a protein does not contain amino acid residues encoded by vector sequences; that is the native protein contains only those amino acids found in the protein as it commonly occurs in nature. A native protein may be produced by recombinant means or may be isolated from a naturally occurring source.

The term "Southern blot," refers to the analysis of DNA on agarose or acrylamide gels to fractionate the DNA according to size followed by transfer of the DNA from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized DNA is then probed with a labeled probe to detect DNA species complementary to the probe used. The DNA may be cleaved with restriction enzymes prior to electrophoresis. Following electrophoresis, the DNA may be partially depurinated and denatured prior to or during transfer to the solid support. Southern blots are a standard tool of molecular biologists (J. Sambrook *et al., Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, NY, pp 9.31-9.58 [1989]).

The term "Northern blot," as used herein refers to the analysis of RNA by electrophoresis of RNA on agarose gels to fractionate the RNA according to size followed by transfer of the RNA from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized RNA is then probed with a labeled probe to detect RNA species complementary to the probe used. Northern blots are a standard tool of molecular biologists (J. Sambrook, *et al.*, *supra*, pp 7.39-7.52 [1989]).

The term "Western blot" refers to the analysis of protein(s) (or polypeptides) immobilized onto a support such as nitrocellulose or a membrane. The proteins are run on acrylamide gels to separate the proteins; followed by transfer of the protein from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized

proteins are then exposed to antibodies with reactivity against an antigen of interest. The binding of the antibodies may be detected by various methods, including the use of radiolabelled antibodies.

The term "antigenic determinant" as used herein refers to that portion of an antigen that makes contact with a particular antibody (*i.e.*, an epitope). When a protein or fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies that bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as antigenic determinants. An antigenic determinant may compete with the intact antigen (*i.e.*, the "immunogen" used to elicit the immune response) for binding to an antibody.

The term "transgene" as used herein refers to a foreign, heterologous, or autologous gene that is placed into an organism by introducing the gene into newly fertilized eggs or early embryos. The term "foreign gene" refers to any nucleic acid (*e.g.*, gene sequence) that is introduced into the genome of an animal by experimental manipulations and may include gene sequences found in that animal so long as the introduced gene does not reside in the same location as does the naturally-occurring gene. The term "autologous gene" is intended to encompass variants (*e.g.*, polymorphisms or mutants) of the naturally occurring gene. The term transgene thus encompasses the replacement of the naturally occurring gene with a variant form of the gene.

As used herein, the term "vector" is used in reference to nucleic acid molecules that transfer DNA segment(s) from one cell to another. The term "vehicle" is sometimes used interchangeably with "vector."

The term "expression vector" as used herein refers to a recombinant DNA molecule containing a desired coding sequence and appropriate nucleic acid sequences necessary for the expression of the operably linked coding sequence in a particular host organism. Nucleic acid sequences necessary for expression in prokaryotes usually include a promoter, an operator (optional), and a ribosome binding site, often along with other sequences. Eukaryotic cells are known to utilize promoters, enhancers, and termination and polyadenylation signals.

As used herein, the term "host cell" refers to any eukaryotic or prokaryotic cell (*e.g.*, bacterial cells such as *E. coli*, CHO cells, yeast cells, mammalian cells, avian cells,

amphibian cells, plant cells, fish cells, and insect cells), whether located *in vitro* or *in vivo*. For example, host cells may be located in a transgenic animal.

The terms "transfection" and "transformation" as used herein refer to the introduction of foreign DNA into cells (e.g. eukaryotic and prokaryotic cells). Transfection may be accomplished by a variety of means known to the art including calcium phosphate-DNA co-precipitation, DEAE-dextran-mediated transfection, polybrene-mediated transfection, electroporation, microinjection, liposome fusion, lipofection, protoplast fusion, retroviral infection, and biolistics.

The term "stable transfection" or "stably transfected" refers to the introduction and integration of foreign DNA into the genome of the transfected cell. The term "stable transfectant" refers to a cell that has stably integrated foreign DNA into the genomic DNA.

The term "transient transfection" or "transiently transfected" refers to the introduction of foreign DNA into a cell where the foreign DNA fails to integrate into the genome of the transfected cell. The foreign DNA persists in the nucleus of the transfected cell for several days. During this time the foreign DNA is subject to the regulatory controls that govern the expression of endogenous genes in the chromosomes. The term "transient transfectant" refers to cells that have taken up foreign DNA but may have failed to integrate this DNA.

The term "calcium phosphate co-precipitation" refers to a technique for the introduction of nucleic acids into a cell. The uptake of nucleic acids by cells is enhanced when the nucleic acid is presented as a calcium phosphate-nucleic acid co-precipitate. The original technique of Graham and van der Eb (Graham and van der Eb, Virol., 52:456 [1973]), has been modified by several groups to optimize conditions for particular types of cells. The art is well aware of these numerous modifications.

A "composition comprising a given polynucleotide sequence" as used herein refers broadly to any composition containing the given polynucleotide sequence. The composition may comprise an aqueous solution. Compositions comprising polynucleotide sequences encoding, for example, a variant Fc region or fragments thereof may be employed as hybridization probes. In this case, variant Fc region encoding polynucleotide sequences are typically employed in an aqueous solution containing salts

(*e.g.*, NaCl), detergents (*e.g.*, SDS), and other components (*e.g.*, Denhardt's solution, dry milk, salmon sperm DNA, etc.).

The term "test compound" or "candidate compound" refer to any chemical entity, pharmaceutical, drug, and the like that can be used to treat or prevent a disease, illness, sickness, or disorder of bodily function, or otherwise alter the physiological or cellular status of a sample. Test compounds comprise both known and potential therapeutic compounds. A test compound can be determined to be therapeutic by screening using the screening methods of the present invention. A "known therapeutic compound" refers to a therapeutic compound that has been shown (*e.g.*, through animal trials or prior experience with administration to humans) to be effective in such treatment or prevention.

As used herein, the term "response," when used in reference to an assay, refers to the generation of a detectable signal (*e.g.*, accumulation of reporter protein, increase in ion concentration, accumulation of a detectable chemical product).

As used herein, the term "reporter gene" refers to a gene encoding a protein that may be assayed. Examples of reporter genes include, but are not limited to, luciferase (*See, e.g.*, deWet *et al.*, Mol. Cell. Biol. 7:725 [1987] and U.S. Pat Nos., 6,074,859, incorporated herein by reference), green fluorescent protein (*e.g.*, GenBank Accession Number U43284; a number of GFP variants are commercially available from CLONTECH Laboratories, Palo Alto, CA), chloramphenicol acetyltransferase, β-galactosidase, alkaline phosphatase, and horseradish peroxidase.

As used herein, the terms "computer memory" and "computer memory device" refer to any storage media readable by a computer processor. Examples of computer memory include, but are not limited to, RAM, ROM, computer chips, digital video disc (DVDs), compact discs (CDs), hard disk drives (HDD), and magnetic tape.

As used herein, the term "computer readable medium" refers to any device or system for storing and providing information (*e.g.*, data and instructions) to a computer processor. Examples of computer readable media include, but are not limited to, DVDs, CDs, hard disk drives, magnetic tape and servers for streaming media over networks.

As used herein, the phrase "computer readable medium encodes a representation" of a nucleic acid or amino acid sequence, refers to computer readable medium that has stored thereon information, that when delivered to a processor, allows the sequence of the

nucleic or amino acid sequence to be displayed to a user (*e.g.* printed out or presented on a display screen).

As used herein, the terms "processor" and "central processing unit" or "CPU" are used interchangeably and refer to a device that is able to read a program from a computer memory (*e.g.*, ROM or other computer memory) and perform a set of steps according to the program.

As used herein, the numbering of amino acid residues in an immunoglobulin heavy chain uses the EU index format as in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991), expressly incorporated herein by reference. The "EU index format as in Kabat" refers to the residue numbering of the human IgG1 EU antibody.

As used herein a "parent polypeptide" is a polypeptide comprising an amino acid sequence that may be changed or altered (e.g. an amino acid substitution, addition or deletion is made) to produce a variant. In preferred embodiments, the parent polypeptide comprises at least a portion of a naturally occurring Fc region or an Fc region with amino acid sequence modifications (e.g., additions, deletions, and/or substitutions). In some embodiments, variants that are shorter or longer than the parent polypeptide are specifically contemplated. In particularly preferred embodiments, the parent polypeptide differs in function (e.g. effector function, binding, etc.) as compared to a variant.

As used herein, the term "variant of a parent polypeptide" refers to a peptide comprising an amino acid sequence that differs from that of the parent polypeptide by at least one amino acid modification. In certain embodiments, the variant comprises at least a portion of an Fc region (e.g. at least 40%, 50%, 75%, or 90% or an Fc region). In preferred embodiments, the variant comprises an Fc region of a parent polypeptide with at least one amino acid modification.

As used herein, the term "Fc region" refers to a C-terminal region of an immunoglobulin heavy chain (e.g., as shown in Figure 1). The "Fc region" may be a native sequence Fc region or a variant Fc region. Although the generally accepted boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. In some

embodiments, variants comprise only portions of the Fc region and can include or not include the carboxyl-terminus. The Fc region of an immunoglobulin generally comprises two constant domains, CH2 and CH3, as shown, for example, in Figure 1. In some embodiments, variants having one or more of the constant domains are contemplated. In other embodiments, variants without such constant domains (or with only portions of such constant domains) are contemplated.

As used herein, the "CH2 domain" (also referred to as"Cγ2"domain) generally comprises the stretch of residues that extends from about amino acid 231 to about amino acid 340 in an Fc region (e.g. in the human IgG Fc region). The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule.

As used herein, the "CH3 domain" (also referred to as"Cγ3"domain) generally comprises the stretch of residues C-terminal to a CH2 domain in an Fc region (e.g., from about amino acid residue 341 to about amino acid residue 447 of a human IgG Fc region).

As used herein, an Fc region may possess "effector functions" that are responsible for activating or diminishing a biological activity (e.g. in a subject). Examples of effector functions include, but are not limited to: C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor; BCR), etc. Such effector functions may require the Fc region to be combined with a binding domain (e.g. an antibody variable domain) and can be assessed using various assays (e.g. Fc binding assay, ADCC assays, CDC assays, target cell depletion from whole or fractionated blood samples, etc.).

As used herein the term "native sequence Fc region" or “wild type Fc region” refers to an amino acid sequence that is identical to the amino acid sequence of an Fc region commonly found in nature. Exemplary native sequence human Fc regions are shown in Figure 2 and include a native sequence human IgG1 Fc region (f and a,z allotypes); native sequence human IgG2 Fc region; native sequence human IgG3 Fc region; and native sequence human IgG4 Fc region as well as naturally occurring variants

thereof. Native sequence murine Fc regions are also shown in Figure 2. Other sequences are contemplated and are readily obtained from various web sites (e.g., NCBI's web site).

As used herein, the term "variant Fc region" refers to amino acid sequence that differs from that of a native sequence Fc region (or portions thereof) by virtue of at least one amino acid modification (e.g., substitution, insertion, or deletion), including heterodimeric variants in which the heavy chain subunit sequences may differ from one another. In preferred embodiments, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region (e.g. from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region). In preferred embodiments, variant Fc regions will possess at least about 80% homology with a native sequence Fc region, preferably at least about 90% homology, and more preferably at least about 95% homology.

As used herein, the term "homology", when used in reference to amino acid sequences, refers to the percentage of residues in an amino acid sequence variant that are identical with the native amino acid sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology.

The term "Fc region-containing polypeptide" refers to a polypeptide, such as an antibody or immunoadhesin (see definitions below), which comprises an Fc region.

The terms "Fc receptor" and "FcR" are used to describe a receptor that binds to an Fc region (e.g. the Fc region of an antibody or antibody fragment). The term includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus.

As used herein, the phrase "antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which cytotoxic cells (e.g. nonspecific) that express FcRs (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cells. The primary cells for mediating ADCC, NK cells, express FcγRIII, whereas monocytes express FcγRI, FcγRII and FcγRIII.

As used herein, the phrase "effector cells" refers to leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express at least

FcγRIII and perform an ADCC effector function. Examples of leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils. The effector cells may be isolated from a native source (e.g. from blood).

As used herein, the phrase "whole blood" refers to unfractionated blood samples.

As used herein, a polypeptide variant with "altered" FcR binding affinity or ADCC activity is one which has either enhanced (i.e. increased) or diminished (i.e. reduced) FcR binding activity and/or ADCC activity compared to a parent polypeptide or to a polypeptide comprising a native sequence Fc region. A polypeptide variant which "displays increased binding" to an FcR binds at least one FcR with better affinity than the parent polypeptide. A polypeptide variant which "displays decreased binding" to an FcR, binds at least one FcR with worse affinity than a parent polypeptide. Such variants which display decreased binding to an FcR may possess little or no appreciable binding to an FcR, e.g., 0-20% binding to the FcR compared to a parent polypeptide. A polypeptide variant which binds an FcR with "better affinity" than a parent polypeptide, is one which binds any one or more of the above identified FcRs with higher binding affinity than the parent antibody, when the amounts of polypeptide variant and parent polypeptide in a binding assay are essentially the same, and all other conditions are identical. For example, a polypeptide variant with improved FcR binding affinity may display from about 1.10 fold to about 100 fold (more typically from about 1.2 fold to about 50 fold) improvement (i.e. increase) in FcR binding affinity compared to the parent polypeptide, where FcR binding affinity is determined, for example, in an ELISA assay.

As used herein, an "amino acid modification" refers to a change in the amino acid sequence of a given amino acid sequence. Exemplary modifications include, but are not limited to, an amino acid substitution, insertion and/or deletion. In preferred embodiments, the amino acid modification is a substitution (e.g. in an Fc region of a parent polypeptide).

As used herein, an "amino acid modification at" a specified position (e.g. in the Fc region) refers to the substitution or deletion of the specified residue, or the insertion of at least one amino acid residue adjacent the specified residue. By insertion "adjacent" a

specified residue is meant insertion within one to two residues thereof. The insertion may be N-terminal or C-terminal to the specified residue.

As used herein, an "amino acid substitution" refers to the replacement of at least one existing amino acid residue in a given amino acid sequence with another different "replacement" amino acid residue. The replacement residue or residues may be "naturally occurring amino acid residues" (i.e. encoded by the genetic code) and selected from: alanine (Ala); arginine (Arg); asparagine (Asn); aspartic acid (Asp); cysteine (Cys); glutamine (Gln); glutamic acid (Glu); glycine (Gly); histidine (His); isoleucine (Ile): leucine (Leu); lysine (Lys); methionine (Met); phenylalanine (Phe); proline (Pro); serine (Ser); threonine (Thr); tryptophan (Trp); tyrosine (Tyr); and valine (Val). Substitution with one or more non-naturally occurring amino acid residues is also encompassed by the definition of an amino acid substitution herein. A "non-naturally occurring amino acid residue" refers to a residue, other than those naturally occurring amino acid residues listed above, which is able to covalently bind adjacent amino acid residues (s) in a polypeptide chain. Examples of non-naturally occurring amino acid residues include norleucine, ornithine, norvaline, homoserine and other amino acid residue analogues such as those described in Ellman et al. Meth. Enzym. 202: 301-336 (1991), herein incorporated by reference.

As used herein, the term "amino acid insertion" refers to the incorporation of at least one amino acid into a given amino acid sequence. In preferred embodiments, an insertion will usually be the insertion of one or two amino acid residues. In other embodiments, the insertion includes larger peptide insertions (e.g. insertion of about three to about five or even up to about ten amino acid residues.

As used herein, the term "amino acid deletion" refers to the removal of at least one amino acid residue from a given amino acid sequence.

The term "assay signal" refers to the output from any method of detecting protein-protein interactions, including but not limited to, absorbance measurements from colorimetric assays, fluorescent intensity, or disintegrations per minute. Assay formats could include ELISA, facs, or other methods. A change in the "assay signal" may reflect a change in cell viability and/or a change in the kinetic off-rate, the kinetic on-rate, or both. A "higher assay signal" refers to the measured output number being larger than

another number (e.g. a variant may have a higher (larger) measured number in an ELISA assay as compared to the parent polypeptide). A "lower" assay signal refers to the measured ouput number being smaller than another number (e.g. a variant may have a lower (smaller) measured number in an ELISA assay as compared to the parent polypeptide).

The term "binding affinity" refers to the equilibrium dissociation constant (expressed in units of concentration) associated with each Fc receptor-Fc binding interaction. The binding affinity is directly related to the ratio of the kinetic off-rate (generally reported in units of inverse time, e.g. seconds^{-1}) divided by the kinetic on-rate (generally reported in units of concentration per unit time, e.g. molar /second). In general it is not possible to unequivocally state whether changes in equilibrium dissociation constants are due to differences in on-rates, off-rates or both unless each of these parameters are experimentally determined (e.g., by BIACORE or SAPIDYNE measurements).

As used herein, the term "hinge region" refers to the stretch of amino acids in human IgG1 stretching from Glu216 to Pro230 of human IgG1. Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain S-S bonds in the same positions.

As used herein, the term "lower hinge region" of an Fc region refers to the stretch of amino acid residues immediately C-terminal to the hinge region (e.g. residues 233 to 239 of the Fc region of IgG1).

"C1q" is a polypeptide that includes a binding site for the Fc region of an immunoglobulin. C1q together with two serine proteases, C1r and C1s, forms the complex C1, the first component of the complement dependent cytotoxicity (CDC) pathway.

As used here, the term "antibody" is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity.

As used herein, the term "antibody fragments" refers to a portion of an intact antibody. Examples of antibody fragments include, but are not limited to, linear

antibodies; single-chain antibody molecules; Fc or Fc' peptides, Fab and Fab fragments, and multispecific antibodies formed from antibody fragments. The antibody fragments preferably retain at least part of the hinge and optionally the CH1 region of an IgG heavy chain. In other preferred embodiments, the antibody fragments comprise at least a portion of the CH2 region or the entire CH2 region.

As used herin, the term "functional fragment", when used in reference to a monoclonal antibody, is intended to refer to a portion of the monoclonal antibody that still retains a functional activity. A functional activity can be, for example, antigen binding activity or specificity. Monoclonal antibody functional fragments include, for example, individual heavy or light chains and fragments thereof, such as VL, VH and Fd; monovalent fragments, such as Fv, Fab, and Fab'; bivalent fragments such as $F(ab')_2$; single chain Fv (scFv); and Fc fragments. Such terms are described in, for example, Harlowe and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1989); Molec. Biology and Biotechnology: A Comprehensive Desk Reference (Myers, R.A. (ed.), New York: VCH Publisher, Inc.); Huston et al., Cell Biophysics, 22:189-224 (1993); Pluckthun and Skerra, Meth. Enzymol., 178:497-515 (1989) and in Day, E.D., Advanced Immunochemistry, Second Ed., Wiley-Liss, Inc., New York, NY (1990), all of which are herein incorporated by reference. The term functional fragment is intended to include, for example, fragments produced by protease digestion or reduction of a monoclonal antibody and by recombinant DNA methods known to those skilled in the art.

As used herein, "humanized" forms of non-human (e.g., murine) antibodies are antibodies that contain minimal sequence, or no sequence, derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are generally made to

further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a nonhuman immunoglobulin and all or substantially all of the FR residues are those of a human immunoglobulin sequence. The humanized antibody may also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Examples of methods used to generate humanized antibodies are described in U.S. Pat. 5,225,539 to Winter et al. (herein incorporated by reference).

As used herein, the term "hypervariable region" refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (i.e. residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (i.e. residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk J. Mol. Biol. 196: 901-917 (1987)). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as defined herein.

As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding domain of a heterologous "adhesin" protein (e.g. a receptor, ligand or enzyme) with an immunoglobulin constant domain. Structurally, immunoadhesins comprise a fusion of the adhesin amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site (antigen combining site) of an antibody (i.e. is "heterologous") with an immunoglobulin constant domain sequence.

As used herein, the term "ligand binding domain" refers to any native receptor or any region or derivative thereof retaining at least a qualitative ligand binding ability of a corresponding native receptor. In certain embodiments, the receptor is from a cell-surface polypeptide having an extracellular domain that is homologous to a member of the

immunoglobulin supergenefamily. Other receptors, which are not members of the immunoglobulin supergenefamily but are nonetheless specifically covered by this definition, are receptors for cytokines, and in particular receptors with tyrosine kinase activity (receptor tyrosine kinases), members of the hematopoietin and nerve growth factor receptor superfamilies, and cell adhesion molecules (e.g. E-, L-, and P- selectins).

As used herein, the term "receptor binding domain" refers to any native ligand for a receptor, including cell adhesion molecules, or any region or derivative of such native ligand retaining at least a qualitative receptor binding ability of a corresponding native ligand.

As used herein, the term "antibody-immunoadhesin chimera" comprises a molecule that combines at least one binding domain of an antibody with at least one immunoadhesin. Examples include, but are not limited to, the bispecific CD4-IgG chimeras described in Berg et al., PNAS (USA) 88:4723-4727 (1991) and Charnow et al., J. Immunol., 153:4268 (1994), both of which are hereby incorporated by reference.

As used herein, an "isolated" polypeptide is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In certain embodiments, the isolated polypeptide is purified (1) to greater than 95% by weight of polypeptides as determined by the Lowry method, and preferably, more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-page under reducing or nonreducing conditions using Coomassie blue, or silver stain. Isolated polypeptide includes the polypeptide in situ within recombinant cells since at least one component of the polypeptide's natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by a least one purification step.

As used herein, the term "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

As used herein, the term "disorder" refers to any condition that would benefit from treatment with a polypeptide variant, including chronic and acute disorders or diseases (e.g. pathological conditions that predispose a patient to a particular disorder). In certain embodiments, the disorder is cancer.

As used herein, the terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer.

As used herein, the phrase "HER2-expressing cancer" is one comprising cells which have HER2 receptor protein (e.g., Genebank accession number X03363) present at their cell surface, such that an anti-HER2 antibody is able to bind to the cancer.

As used herein, the term "label" refers to a detectable compound or composition that is conjugated directly or indirectly to a polypeptide. The label may itself be detectable (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

As used herein, the terms "control element", "control sequence" and "regulatory element" refers to a genetic element that controls some aspect of the expression of nucleic acid sequences. For example, a promoter is a regulatory element that facilitates the initiation of transcription of an operably linked coding region. Other regulatory elements include splicing signals, polyadenylation signals, termination signals, etc. Control elements that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

As used herein, nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. In preferred embodiments, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading frame. However enhancers, for example, do not have to be contiguous. Linking may be accomplished, for example, by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers may be used in accordance with conventional practice.

As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

As used herein, "analyte" refers to a substance that is to be analyzed. The preferred analyte is an Fc region containing polypeptide that is to be analyzed for its ability to bind to an Fc receptor.

As used herein, the term "receptor" refers to a polypeptide capable of binding at least one ligand. The preferred receptor is a cell-surface or soluble receptor having an extracellular ligand-binding domain and, optionally, other domains (e.g. transmembrane domain, intracellular domain and/or membrane anchor). A receptor to be evaluated in an assay described herein may be an intact receptor or a fragment or derivative thereof (e.g. a fusion protein comprising the binding domain of the receptor fused to one or more heterologous polypeptides). Moreover, the receptor to be evaluated for its binding

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